# 5. Summary of Data Reported and Evaluation

## 5.1 Exposure data

The use of tobacco dates back to at least 5000 years in the Americas, and, by the 1700s, had spread throughout the world. The major tobacco products are made primarily from the *Nicotiana tabacum* species, but those in Asia and Africa are frequently also made from *N. rustica* and other species. Globally, smokeless tobacco includes a wide variety of commercially and non-commercially prepared products that are used either orally or nasally and are not burned when used. They are consumed by hundreds of millions of people in many regions of the world. The largest number of users of smokeless tobacco live in South-East Asia, particularly in India and Bangladesh. Consumption appears to be increasing in many populations.

Smokeless tobacco products differ in their composition and chemical profile, but they all contain nicotine, which is an addictive substance. The doses of nicotine in smokeless tobacco products, particularly in moist snuff, are manipulated by commercial manufacturers, and levels vary among product types and brands as a result of tobacco-processing techniques. In addition, all smokeless tobacco products expose users to a number of identified carcinogens that arise mainly during post-harvest processing of the tobacco; the most abundant in smokeless tobacco products are tobacco-specific *N*-nitrosamines, *N*-nitrosoamino acids, volatile *N*-nitrosamines and aldehydes. The amounts of *N*-nitrosamine in smokeless tobacco exceed those found in food and cosmetic products by several orders of magnitude. Some smokeless tobacco products also contain high levels of carcinogenic polycyclic aromatic hydrocarbons.

Some health scientists have suggested that smokeless tobacco should be used in smoking cessation programmes and have made implicit or explicit claims that its use would partly reduce the exposure of smokers to carcinogens and the risk for cancer. They also attribute declines in smoking in Sweden to increased consumption of moist snuff; these claims, however, are not supported by the available evidence.

Particular types of smokeless tobacco are banned or regulated in parts of the world, but, in many areas, either product regulation is non-existent or the degree of enforcement of established regulations is uncertain.

## 5.2 Human carcinogenicity data

*Oral cancer*

Several studies in various countries have identified the use of smokeless tobacco as a cause of oral cancer. A case–control study in the USA (North Carolina) that investigated large numbers of smokeless tobacco users who did not smoke found that the risk for oral

cancer was strongly associated with the use of snuff among nonsmokers who did not drink alcoholic beverages. A dose–response relationship was observed between increasing duration of use and the risk for cancers of the gum and buccal mucosa.

Additional strong evidence is available from two large case–control studies — one from India in 1962 and one from Pakistan in 1977 — that reported two- to 14-fold increases in the risk for oral cancer among chewers of tobacco (or tobacco plus lime) who were not betel-quid chewers and were also nonsmokers. Another case–control study from Pakistan in 2000 on users of *naswar* reported a nearly 10-fold increase in the risk for oral cancer after adjusting for tobacco smoking and alcoholic beverage consumption. An additional case–control study from Nagpur, India, reported an eightfold increase in risk for smokeless tobacco use among nonsmokers and also reported a 15-fold increase in risk for all oral cancers combined for those who use materials that contain tobacco to clean their teeth, after adjusting for tobacco smoking, alcoholic beverage consumption, occupation and tobacco chewing.

Two population-based case–control studies on snuff use — one on head and neck cancer and one on oral cancer — were conducted in southern and northern parts of Sweden. The study from southern Sweden found no significant association between snuff use and the risk for head and neck cancer, either for all sites combined or when restricted to cancers of the oral cavity. When the analysis was restricted to men with no history of smoking, there was a nearly fivefold elevated risk for head and neck cancer associated with snuff use. The small sample size precluded a separate site-specific analysis for oral cancer among those who never smoked. The study conducted in northern Sweden investigated cancer of the oral cavity in relation to snuff use and controlled for alcoholic beverage use and tobacco smoking. Overall, this study did not suggest an association between snuff use and oral cancer. However, some relevant subgroups (e.g. those who never smoked, cases of lip cancer) had increased relative risks that were of borderline statistical significance. In both studies, the risks for oral cancer among former snuff users were increased with borderline statistical significance.

One cohort study from the USA reported a non-significant increased risk for oral cancer among those who never smoked but used smokeless tobacco. Less confidence can be placed on two other cohort studies from the USA and one from Norway that did not report an increase in risk, because the number of cases was small or the effect estimates were not controlled for tobacco smoking.

Additional support for a causal association derives from four case–control studies in North America that also showed a relationship between the use of smokeless tobacco and oral or oral and pharyngeal cancer. These studies addressed potential confounding by tobacco smoking through stratification by examining nonsmokers only or by statistical adjustment. However, they were based on small numbers and internal consistency in the results was not assessed.

In a number of regions across the world, supporting evidence for an association between the oral use of tobacco and increased risk for oral cancer is based on studies that have reported high prevalences of users of these products in case series of oral cancer and reports

of cancers that developed at anatomical sites where the tobacco was placed. In studies that had some methodological limitations, high rates of oral cancer have been reported in regions that had high prevalences of smokeless tobacco use, e.g. among *toombak* users in Sudan and among *naswar* and *shammah* users in central Asia and Saudi Arabia.

Cross-sectional studies in many countries have demonstrated strong associations between smokeless tobacco use (after accounting for confounding factors) and precancerous lesions such as oral leukoplakia.

The studies from the USA, Asia and Africa — in particular, one study from the USA and four studies from South Asia — provide sufficient evidence for a causal association of smokeless tobacco use with oral cancer. The Swedish studies are not inconsistent with positive studies in other regions for various reasons. First, variations in magnitudes of risk across studies may be due to differences in tobacco species and tobacco processing or in practices that include amounts used, years of use or keeping the tobacco in the mouth for long periods; in addition, variations in oral hygiene status or individual susceptibility factors may also play a role. Second, in one Swedish study, positive findings were observed in the subgroup of those who had never smoked and, in both Swedish studies, risks were elevated in former users, which might be expected if the presence of oral precancerous lesions led to cessation of the use of smokeless tobacco.

*Oesophageal cancer*

A fivefold increase in risk for oesophageal cancer among chewers of tobacco leaves (locally called *chada*) was reported among nonsmokers (adjusted for alcoholic beverage use) and among non-alcoholic beverage drinkers (adjusted for smoking) in a case–control study from Assam, India. Similar levels of risk were observed among men and women when they were analysed separately.

In a Swedish case–control study, only a modest increase in risk was observed overall, but a higher increase in risk was found for long-term users. This study also reported a dose–response with intensity of use, although there was no increased risk in the highest category. A cohort study from Norway found a modest, statistically non-significant increase in risk. Another Swedish case–control study of head and neck cancer reported only a very modest increase in risk for oesophageal cancer and a case–control study in the USA reported no effect.

*Pancreatic cancer*

Two case–control studies from the USA and two cohort studies, one from the USA and one from Norway, have reported positive associations between the use of smokeless tobacco and pancreatic cancer. In one case–control study in the USA, a statistically significant elevated risk for pancreatic cancer was observed among those who had never smoked and long-term quitters. In the other case–control study among lifelong nonsmokers in the USA, an elevated risk among users of more than 2.5 ounces [~70 g] per week was reported. One cohort study of men in Norway found an excess risk for pancreatic cancer among those who had ever used smokeless tobacco after controlling for smoking; however,

in a stratified analysis, the excess risk was confined to smokers. The Lutheran Brotherhood cohort study found an excess risk of borderline significance in those who had ever used smokeless tobacco, taking into account smoking and alcoholic beverage consumption. The evidence on dose–response relationships is restricted to one study from the USA which found an increased risk only among heavy users of smokeless tobacco.

*Other cancers*

Studies on cancers at other sites did not provide conclusive evidence of a relationship with smokeless tobacco use.

*Nasal use*

Studies on nasal use of snuff did not provide conclusive evidence of a relationship with cancer.

### 5.3 Animal carcinogenicity data

In two studies, squamous-cell carcinomas and papillomas of the oral and nasal cavities and forestomach and undifferentiated sarcomas of the lip developed with a significantly increased incidence in rats that had received moist snuff tobacco repeatedly applied to a surgically created oral canal. When snuff-treated rats were pretreated with 4-nitroquinoline *N*-oxide, an increased incidence of sarcomas of the lip was observed. In another experiment, benign and malignant epithelial tumours of the oral cavity developed in rats when snuff tobacco, water-extracted snuff tobacco or snuff tobacco enriched with its own aqueous extract was applied to a surgically created oral canal. However, the increase in tumour incidence did not achieve statistical significance. In addition, snuff tobacco was tested for carcinogenicity in rats by topical administration in a surgically-created oral canal alone or in combination with herpes simplex virus type 1 infection. Squamous-cell carcinomas of the oral cavity were observed in the group that received both treatments, but this result was not statistically significant.

Rats given tobacco extracts by gavage showed a statistically non-significantly increased incidence of forestomach papillomas and lung adenomas, and rats on a vitamin A-deficient diet given the same tobacco extract developed a high incidence of forestomach papillomas and pituitary adenomas. Weekly applications of snuff tobacco to the oral mucosa caused no tumours in rats of either sex. Subcutaneous injection of ethanol extracts of snuff tobacco to rats did not produce an increase in tumour incidence. Aqueous snuff tobacco extracts and snuff tobacco extracts enriched to 10-fold their natural concentrations of tobacco-specific nitrosamines were tested by repeatedly swabbing the lips and oral cavities of rats. A small, statistically non-significant increase in the incidence of lung adenomas and papillomas of the oral cavity occurred in rats treated with preparations enriched in tobacco-specific nitrosamines, but non-enriched snuff tobacco extracts alone produced no tumours of either the oral cavity or lung.

In one experiment, inoculation of herpes simplex virus-1 or -2 into the cheek pouches of hamsters followed by repeated application of snuff tobacco into the cheek pouches resulted in a high incidence of invasive squamous-cell carcinomas at the site of application. No tumours developed in cheek pouches treated with inoculations of virus alone or in those treated with snuff alone or in controls. In one experiment, snuff tobacco suspended in liquid paraffin and administered repeatedly to hamster cheek pouches caused forestomach papillomas but no tumours at the site of application. Hamsters given tobacco alone or in combination with alcohol into the cheek pouch developed a low and statistically non-significantly increased incidence of forestomach papillomas, but no tumours developed in the treated cheek pouches. Several studies in hamsters in which snuff tobacco alone or in combination with calcium hydroxide was administered as single or repeated applications into the cheek pouch or fed in the diet gave negative results or yielded inadequate data for evaluation.

In a study in which *mishri* was fed in the diet, an increase in the incidence of forestomach papillomas was observed in mice, hamsters and rats. Malignant tumours of the lung and stomach papillomas developed in rats maintained on a vitamin A-deficient diet and given *mishri* by gavage. In one study, repeated application of *mishri* extract to mouse skin resulted in the development of skin papillomas in some mice and one squamous-cell carcinoma of the skin.

In a two-stage mouse skin assay, applications of tobacco extract followed by promotion with croton oil induced papillomas and squamous-cell carcinomas of the skin. In another two-stage mouse skin assay, application of *bidi* tobacco extracts following initiation by 7,12-dimethylbenz[*a*]anthracene resulted in papillomas.

Available studies on *naswar* were inadequate for evaluation.

### 5.4 Other relevant data

Tobacco-specific nitrosamines, the most abundant strong carcinogens in smokeless tobacco products, nicotine and cotinine have been detected in the saliva of snuff dippers and tobacco chewers in many studies around the world. Levels of tobacco-specific nitrosamines in saliva are remarkably high in Sudanese users of oral snuff (*toombak*). Adducts of tobacco-specific nitrosamines to haemoglobin — via analysis of an alkaline hydrolysis product — have been explored as biomarkers of exposure to smokeless tobacco and were found in snuff dippers and nasal snuff users in several studies. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol, a metabolic product of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and a glucuronidation product of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol are very useful and specific biomarkers of tobacco use and provide a good approximation of carcinogenic dose. These substances have been found in the urine of smokeless tobacco users in numerous studies, and the *toombak* users in the Sudan showed exceptionally high concentrations (excretion of up to 0.4 mg daily).

*N*′-Nitrosonornicotine, *N*′-nitrosoanabasine and *N*′-nitrosoanatabine and their respective glucuronides have been detected in the urine of smokeless tobacco users at significantly higher levels than in non-users.

In addition to tobacco-specific nitrosamines, tobacco also contains secondary and tertiary amines that can be nitrosated by reaction with available nitrite in the saliva or in the stomach of tobacco chewers (endogenous nitrosation). This process is enhanced by bacteria in dental plaque and by the acidic environment in the stomach (many chewers swallow the chewed tobacco).

In humans, the absorption of nicotine from smokeless tobacco products is slower than that from tobacco products used for smoking, but overall equivalent plasma levels of nicotine are achieved. The absorption of nicotine is largely dependent on the pH of the product–buccal interface. Other factors, such as the quantity of smokeless tobacco used, product flux, nicotine content of the product and the length of time that the product is in contact with the buccal membrane also determine the extent and rapidity of absorption. Once nicotine is absorbed at the buccal membrane, it enters the systemic circulation (avoiding first-pass hepatic metabolism) and is rapidly distributed throughout the body. Nicotine is cleared from the blood by hepatic metabolism to cotinine, *trans*-3′-hydroxycotinine and other products that are excreted in the urine. Considerable quantities of nicotine can be absorbed rapidly from smokeless tobacco products, which leads to reinforced feelings of euphoria, re-administration, neuroadaptation and compulsive use that are the hallmark characteristics of drugs that produce dependence.

Since malnutrition is a problem in many countries where the use of smokeless tobacco is highly prevalent, experimental studies in rats have focused on dietary modulation of the effects of smokeless tobacco and have shown that smokeless tobacco is more toxic to rats fed vitamin- or protein-deficient diets than to animals fed healthy diets. Remarkably, the activity of phase I enzymes involved in the bioactivation of xenobiotics was increased, while that of detoxification enzymes was decreased after chronic exposure to smokeless tobacco products in the diet.

In experimental systems, exposure to smokeless tobacco products was associated with the generation of reactive oxygen species, modulation of inflammatory mediators, inhibition of collagen synthesis and impairment of DNA repair capacity.

Smokeless tobacco products deliver nicotine in quantities and at rates that cause psychoactive effects, which eventually lead to tolerance and addiction. All of the currently recognized criteria to establish that a drug produces dependence are fulfilled in the case of smokeless tobacco products, which are psychoactive and induce a compulsive pattern of use. On discontinuation of use, drug craving and other signs of drug withdrawal are evident. Furthermore, there is a high rate of relapse among people who attempt to quit smokeless tobacco products. The effects of the use and discontinuation of use of smokeless tobacco products are similar to those of nicotine delivered through cigarette smoking. It was concluded that addiction to smokeless tobacco is analogous to addiction to nicotine.

The pathology of soft-tissue lesions in the mouth associated with the use of smokeless tobacco indicates features of premalignancy and neoplasia at the site of application. In

studies in human volunteers, application of smokeless tobacco products produced morphological changes, and white and erythematous lesions of oral mucosa. In experimental systems *in vitro*, smokeless tobacco products have been shown to affect inflammatory mediators, cell proliferation and apoptosis.

The evidence on the risk for cardiovascular disease from smokeless tobacco use is limited. Three cohort studies observed statistically significant increased risks for mortality from cardiovascular disease, with increased risks for both coronary heart disease and stroke, while four other cohort and case–control studies observed no significant increased risks for particular cardiovascular disease outcomes. Most of these studies suffer from important limitations. Evidence on most subclinical cardiovascular end-points is similarly inconclusive, although smokeless tobacco clearly causes acute increases in blood pressure and heart rate. A small increase in the risk for cardiovascular disease from smokeless tobacco use is certainly possible and, because of the high background rates of cardiovascular disease, even a small increase in relative risk could represent a large public health impact in countries that have a high prevalence of smokeless tobacco use.

The data on smokeless tobacco use and insulin resistance, glucose intolerance and diabetes are limited and the results are inconsistent. Effects on insulin sensitivity, glucose tolerance and the risk for diabetes from smokeless tobacco use are plausible, however, based on some positive results seen in the available studies of smokeless tobacco and nicotine. Diabetic smokeless tobacco users, in particular, may be at increased risk for aggravated insulin resistance.

The use of smokeless tobacco causes reproductive and developmental toxicity. In humans, the use of smokeless tobacco during pregnancy increases the risks for pre-eclampsia and premature birth, causes increased placental weight and reduces mean birth weight. Smokeless tobacco use by men causes reduced semen volume, reduced sperm count, reduced sperm motility and an increased frequency of abnormal spermatozoa.

In pregnant mice, extracts of moist snuff caused increased placental weights, reduced fetal weights, retarded fetal skeletal ossification and increased the rate of fetal resorption. Infant mice exposed translactationally to smokeless tobacco extracts had depressed levels of hepatic glutathione *S*-transferase, depressed hepatic thiol content and increased cytochrome P450 levels.

Elevated micronucleus formation, sister chromatid exchange and chromosomal aberrations have been reported in the oral exfoliated cells of consumers of smokeless tobacco, and TP53 protein accumulation and mutations have been reported in their oral premalignant lesions and squamous-cell carcinomas. These mutations include G→A and C→T transitions and G→T transversions. Mutations in *H-RAS* and $p21^{waf1}$ and other alterations in gene expression were also observed in oral premalignant lesions and squamous-cell carcinomas of smokeless tobacco consumers.

In a study of dietary modulation, the urine of rats that received an intraperitoneal dose of smokeless tobacco extract was mutagenic in *Salmonella typhimurium*. The level of urinary mutagenicity was higher in rats fed vitamin- and protein-deficient diets than in animals fed a normal diet.

Numerous studies in different types of prokaryotic and eukaryotic cells *in vitro* have reported the mutagenicity and clastogenicity of aqueous and organic extracts of a variety of smokeless tobacco products, including Yemeni snuff, Swedish moist oral snuff and various types of American and Indian chewing tobacco.

A few studies have examined the effects of smokeless tobacco and tobacco-specific nitrosamines on viral infection. These substances enhanced cell transformation by herpes simplex virus type 1 and inhibited replication of the virus in the oral cavity.

## 5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of smokeless tobacco. Smokeless tobacco causes cancers of the oral cavity and pancreas.

There is *sufficient evidence* in experimental animals for the carcinogenicity of moist snuff.

### Overall evaluation

Smokeless tobacco is *carcinogenic to humans (Group 1).*